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Ultrastructural comparison between the tongue of two reptilian species endemic in Egyptian fauna; Bosc's fringe-toed lizard *Acanthodactylus boskianus* and Sinai fan-fingered gecko *Ptyodactylus guttatus*

Doaa I. Gewily[1] | Fatma A. Mahmoud[2] | Samy A. Saber[3] | Boshra A. ElSalkh[1] | Asmaa A. El-Dahshan[1] | Mohamed M. A. Abumandour[4] | Ramadan M. Kandyel[5] | Ali G. Gadel-Rab[6]

[1]Department of Zoology, Faculty of Science (Girls), Al-Azhar University, Cairo, Egypt
[2]Department of Zoology, Faculty of Science, Assuit University, Assiut, Egypt
[3]Department of Zoology, Faculty of Science, Al-Azhar University, Cairo, Egypt
[4]Department of Anatomy and Embryology, Faculty of Veterinary Medicine, Alexandria University, Alexandria, Egypt
[5]Department of Zoology, Faculty of Science, Tanta University, Tanta, Egypt
[6]Department of Zoology, Faculty of Science, Al-Azhar University, Assiut, Egypt

Correspondence
Mohamed M. A. Abumandour, Assistant Professor of Anatomy and Embryology, Faculty of Veterinary Medicine, Alexandria University, Egypt. Mail address of the corresponding author: Anatomy and Embryology Department, Faculty of Veterinary Medicine, Alexandria, Egypt, Post Box: 22785.
Email: m.abumandour@alexu.edu.eg

Ali G. Gadel-Rab, Lecturer of Vertebrates, Faculty of Science, Al-Azhar University, Assiut, Egypt.
Email: aligamal200992@yahoo.com

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Abstract

The current observations focused on the ultrastructure comparison between the tongue of two reptile species endemic the Egyptian fauna; Bosc's fringe-toed lizard *Acanthodactylus boskianus* and Sinai fan-fingered gecko *Ptyodactylus guttatus* to exhibit the relationship between the lingual epithelium and its function according to their specific feeding strategy. *A. boskianus* possessed triangular elongated tongue with bifurcated tapering apex and wide base while; the *P. guttatus* had a triangular flattened tongue with conical shallow bifurcated apex and broad base. The ventral surface of the lingual apex of *A. boskianus* had transverse while in *P. guttatus* had two oval pads and median ventral groove. Both surfaces of the tongue of both examined species are covered by stratified squamous epithelium with great variability of degree of keratinization. The dorsal epithelium formed flattened and conical filiform papillae in *A. boskianus*, while in *P. guttatus* formed cylindrical papillae, conical, and tall filiform ones. Few taste buds are observed on the fore-tongue but increase on the mid-tongue of *A. boskianus*, while in *P. guttatus*, numerous taste buds are distributed on the fore-tongue and mid-tongue. Both surfaces of the laryngeal mound of both examined species provided with numerous of cilia and orifices of laryngeal gland. The present results confirmed that the tongue of *A. boskianus* acts as a chemoreceptor organ to follow pheromone trails of prey and mates. While in *P. guttatus* the tongue may play an important role in the feeding mechanism and act as a chemoreceptor organ.

KEYWORDS
Acanthodactylus boskianus, adaptation, histology, lingual epithelium, *Ptyodactylus guttatus*, SEM

1 | INTRODUCTION

Bosc's fringe-toed lizard *Acanthodactylus boskianus* is a species of lizard that belonged to *Squamata* order, *Lacertidae* family, *Acanthodactylus* Genus (El Din, 2006). This fast-moving lizard endemic in Egyptian fauna and mostly diurnal species and habitat the dry with little vegetated region and depending on the insects such as beetles, flies and some spiders on their feeding. Sinai fan-fingered gecko

Ptyodactylus guttatus (Heyden, 1827) is a species of gecko that belonged to *Squamata* order, *phyllodactylidae* family, *Ptyodactylus* Genus (El Din, 2006). This endemic Egyptian fauna species and depending on the small insects such as flies and some spiders on their feeding.

The specific feeding mechanism is an important factor that determines the success of adaptation process of the different vertebrate-species to their surrounding environment conditions through propagation (Abumandour, 2018; Abumandour & El-Bakary, 2017a; Bels et al., 2019; Montuelle, Herrel, Libourel, Reveret, & Bels, 2009; Reilly, McBrayer, McBrayer, & Miles, 2007). The tongue is considered a key innovation in the evolution of a terrestrial lifestyle as it allows animals to transport food items through the oral cavity (Abumandour & El-Bakary, 2013; Abumandour & El-Bakary, 2017b; Abumandour & El-Bakary, 2019; Herrel, Canbek, Ozelmas, Uyanoglu, & Karakaya, 2005; Iwasaki, 2002; Meyers & Herrel, 2005). The tongue as a hyobranchial system has been coopted for a wide diversity of functions such as prey capture, drinking, breathing, and defensive behaviors (El-Mansi, Al-Kahtani, Abumandour, & Ahmed, 2020; Herrel, Timmermans, & De Vree, 1998; Iwasaki, 2002; Kuo, Munoz, & Irschick, 2019; McClung & Goldberg, 2000; Schwenk, 1995; Schwenk, 2000).

Among the vertebrates, the reptilian tongues are characterized by the presence of the morphological and functional variations among species that reflecting the various functions of each respective tongue (Cooper, 2003; Darwish, 2012; El-Mansi et al., 2020; El-Sayyad, Sabry, Khalifa, Abou-El-Naga, & Foda, 2011; Iwasaki, 2002). Many previous published descriptive studies were depending on the light microscopic examinations to investigate the structure of the tongue surface of the different reptile-species such as (Abbate et al., 2010; Bayoumi, Abd-Elhameed, & Mohamed, 2011; El-Mansi et al., 2020; Mohammed, Mohallal, & Attia, 1998; Santos et al., 2011; Wassif & El-Hawary, 1998), these studies showed a great variety in the lingual morphology and histology, especially variation in the shape of the dorsal and ventral papillae. Apparently, the differences between the tongue surfaces of various reptiles depend on dissimilarities in the diet, the feeding habits, and the handling of the food in the oral cavity (Mohammed, 1992).

The different kinds of the lingual papillae had been observed along tongue's surface of the different reptile's species, each having different morphological structure and shape. The distribution of these lingual papillae has been considered to be related to species-specific feeding habits and vocalization (Park & Lee, 2009). However, that similarities and differences which exhibited in the structure of the lingual epithelium among the different reptile's species is attracted the attention of the present authors. The present authors discuss these modifications that occur in the lingual epithelium with the different feeding mechanism in the two examined reptile-species that endemic in the Egyptian fauna: Bosc's fringe-toed lizard *Acanthodactylus boskianus* and Sinai fan-fingered gecko *Ptyodactylus guttatus*. Since *A. boskianus* (Lizard) is a diurnal insectivorous species foraging in open area with sparse vegetation adopting active search strategy to find food, while, *P. guttatus* (Gecko) is a diurno-nocturnal species, feed on insects adopting "sit and wait" strategy (Saber, Bashandy, Kawashti, & Sadek, 1994).

2 | MATERIALS AND METHODS

2.1 | Animals handling and preparation

Twelve adult Bosc's fringe-toed lizard *Acanthodactylus boskianus* and spotted fan-toed geckos *Ptyodactylus guttatus* were captured alive from their natural habitat in the different regions of Sinai, Egypt. The collected examined reptilian species were kept in the animal holding of the Department of Zoology, Faculty of Science at Assiut University

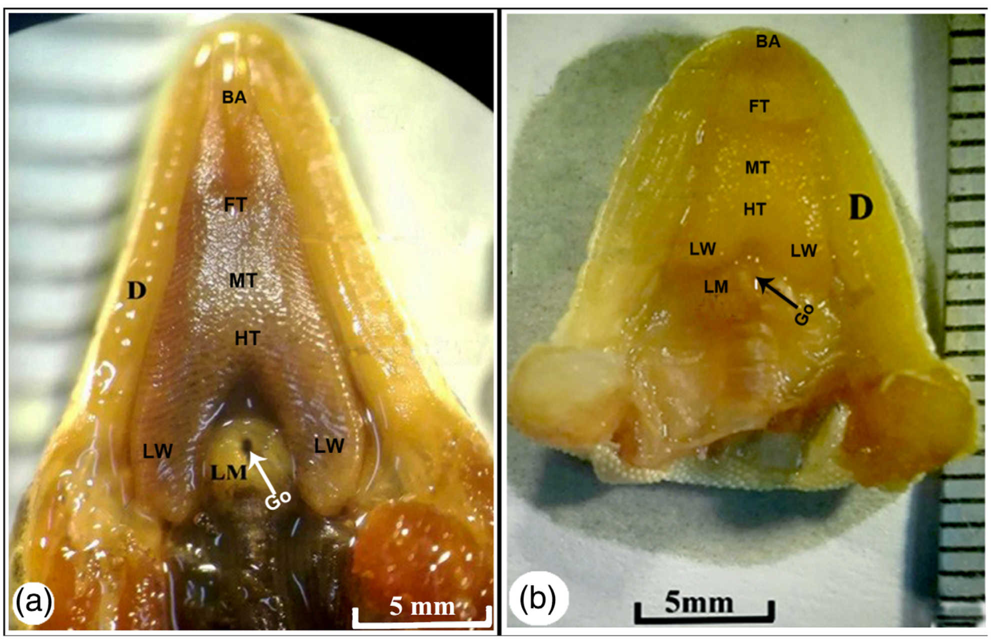

FIGURE 1 Stereomicroscopic images of the anatomical appearance of the tongue in Bosc's fringe-toed lizard *Acanthodactylus boskianus* (View a) and Sinai fan-fingered gecko *Ptyodactylus guttatus* (View b) to show; Fore-tongue (FT) with bifurcated apex (BA), Mid-tongue (MT), Hind-tongue (HT) with lingual wing (LW) and laryngeal mound (LM) with median glottis (Go) and the lower jaw (D) [Color figure can be viewed at wileyonlinelibrary.com]

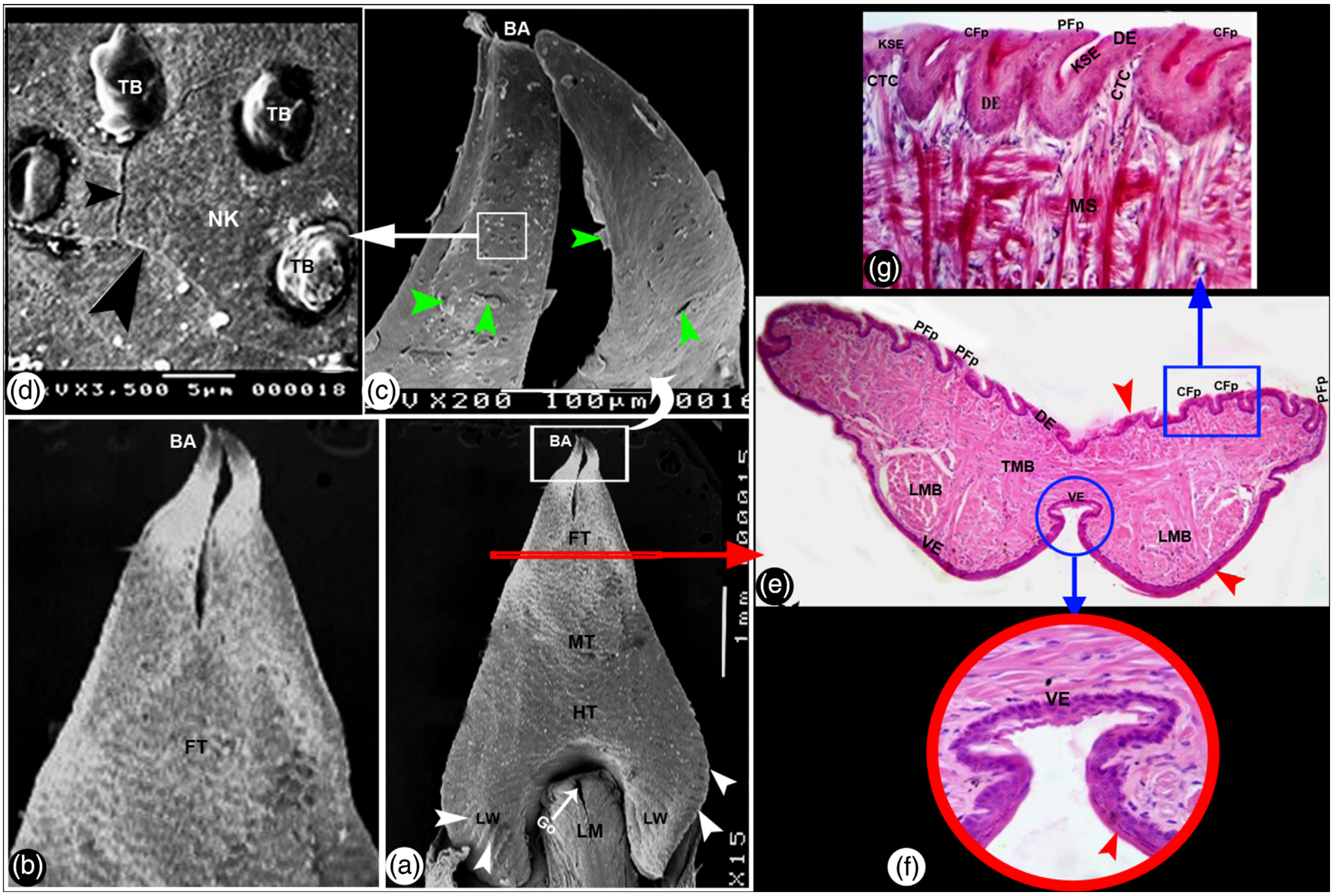

FIGURE 2 Morphological images of the tongue in the Bosc's fringe-toed lizard *Acanthodactylus boskianus*. (View a–d) represent the SEM images of the fore-tongue to show; Fore-tongue (FT) with bifurcated apex (BA), deciduous cells (green arrowhead), taste buds (TB) and intercellular borders cell (black arrowheads). Mid-tongue (MT), Hind-tongue (HT) with lingual wing (LW) and laryngeal mound (LM) with median glottis (Go), transverse arranged lingual papillae (white arrowheads). (View e–g) represent the histological images of the fore-tongue to show the appearance of the flattened conical (CFp) and pointed conical filiform papillae (PFp), keratin layer (red arrowheads) on the dorsal epithelium (DE) that covered by keratinized stratified squamous epithelium (KSE) and ventral epithelium (VE), transverse muscles bundles (TMB), longitudinal muscles bundles (LMB), connective tissue core (CTC). (View e,f) ×40 with H&E stain, while (View g) ×400 with Masson's trichrome stain [Color figure can be viewed at wileyonlinelibrary.com]

and were carefully conditioned. Handling and care of the animals was approved by the Ethical Committee on Animal Experimentation of the Faculty of Science at Assiut University, Egypt. The 12 specimens of each study species were divided into three groups, each of which formed of four specimens. After anesthetization the specimens, the head were separated from the rest of body then carefully dissected the tongue from the surrounding tissue, for example, muscles, fascia, glands, …, etc.

2.2 | For stereomicroscopic examinations

Four tongues from each examined reptilian species were quickly transferred into 10% formalin for 3 weeks and stored in 2% phenoxyethanol for long-term preservation. Tongues were examined under a Wild M3 Stero-microscope (Olympus VM VMF 2x, Eyepiece 10x Stereo Microscope, Japan) to obtain magnified images of the tongue outline. Then, these morphological lingual characterizations were photographed by a digital camera (Canon IXY 325, Japan). The anatomical terminology of tongue according to (Nomina Anatomica Veterinaria, 2017).

2.3 | For scanning electron microscopy examinations

The scanning electron microscopic (SEM) examination and description of the tongue of the two examined reptilian species were carried out on four tongue. Small samples from tongue were put in fixed solution (2% formaldehyde, 1.25% glutaraldehyde in 0.1 M sodium cacodylate buffer) at 4°C and at pH 7.2. Then, the samples were passed in washing process (in 0.1 M sodium cacodylate containing 5% sucrose) then, processed by the tannic acid. Then, the samples were exposed to dehydration by passing in the increasing concentrations of ethanol

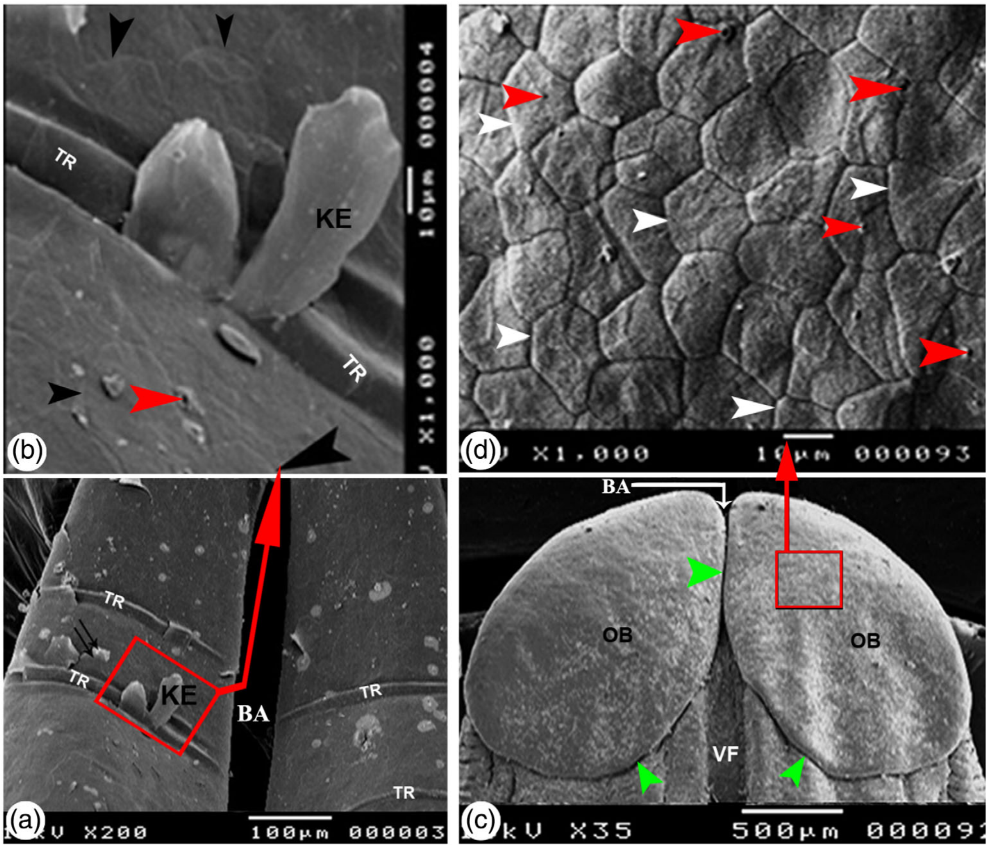

FIGURE 3 SEM images of the ventral surface of the lingual apex of the tongue in the two examined species. (View a,b) represent the ventral surface of the lingual apex of the Bosc's fringe-toed lizard *Acanthodactylus boskianus* showing; The bifurcated apex (BA) covered by desquamated keratin (KE) and shallow intercellular borders (black arrowhead), the detached keratin layer (Double black arrows), transverse retridges (TR) and opening of the lingual glands (red arrowheads). (View c,d) represent the ventral surface of the lingual apex of the Sinai fan-fingered gecko *Ptyodactylus guttatus* showing; the two oval pads (OB) that surrounded by a semicircular shallow groove (green arrowhead), the shallow bifurcation (BA) that continuous posteriorly as the median ventral groove (VF) had pentagonal and hexagonal shapes (white arrowheads) and opening of the lingual glands (red arrowheads) [Color figure can be viewed at wileyonlinelibrary.com]

(15 min in 50, 70, 80, 90, 95, and 100% ethanol). At the end, the samples were dried by putting in the solution formed from the carbon dioxide, then attached to stubs with the colloidal carbon and coated by gold palladium in a sputtering device. Then, samples were examined and photographed with a JEOL JSM 5400IV SEM device, 15 kV, Faculty of Science, Assiut University, Egypt.

2.4 | For histological examinations

For light microscopic investigations, four tongues of both examined reptilian species were fixed in 10% phosphate buffered formalin (pH 7.4), dehydrated in ascending concentrations of ethyl alcohol, cleared in xylene, and embedded in molten paraplast at 58–62°C. Then, samples were sectioned in five microns using Leica rotatory microtome (RM 20352035; Leica Microsystems, Wetzlar, Germany) according to (Suvarna, Layton, & Bancroft, 2013). Then, the samples were stained with Haematoxylin and Eosin (Cook & Stirling, 1994) for examination of the general histology, Masson's Trichrome stain (Masson, 1929) for detect collagen fibers, and periodic acid Schiff's reagent (PAS) (Schumacher, Duku, Katoh, Jörns, & Krause, 2004) to demonstrate the neutral mucin.

3 | RESULTS

The tongue of both examined reptilian species is a dorsoventrally flattened and distinguished into three lingual parts; the fore-tongue, the midtongue, and the hind-tongue. The laryngeal entrance represented by the laryngeal mound with a median longitudinal glottis opening, is located immediately posterior to the lingual wing (Figure 1).

3.1 | Bosc's fringe-toed lizard *Acanthodactylus boskianus*

3.1.1 | Morphological investigation

The tongue of *Acanthodactylus boskianus* is triangular with length (~1.2 mm) about two third the length of mandible (~1.4 mm). The

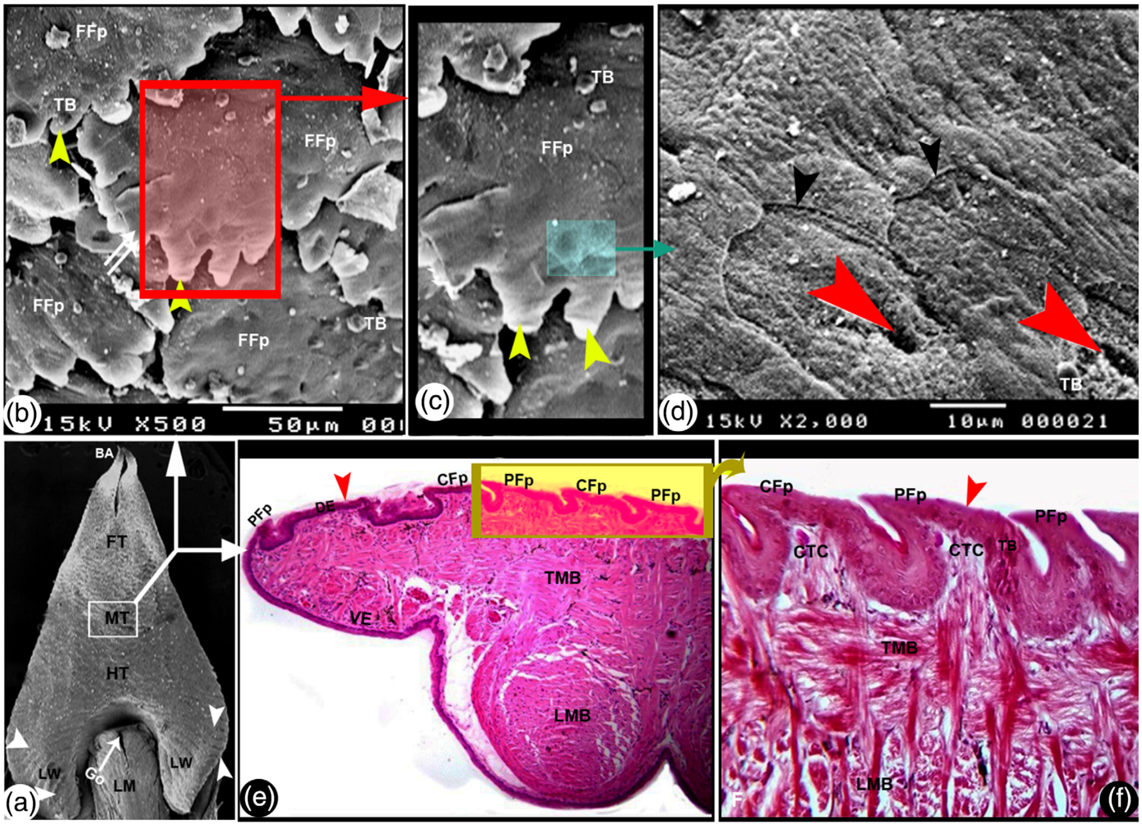

FIGURE 4 Morphological images of the mid-tongue in the Bosc's fringe-toed lizard *Acanthodactylus boskianus*. (View a–d) represent the SEM images showing; to show; Fore-tongue (FT) with bifurcated apex (BA), Mid-tongue (MT), Hind-tongue (HT) with lingual wing (LW) and laryngeal mound (LM) with median glottis (Go) and transverse lingual papillae (white arrowheads), overlap flattened filiform papillae (FFp) with irregular free edge (yellow headarrows), taste buds on the papillary surface (TB), intercellular borders (black arrowhead) with openings of the lingual glands (red arrowheads). (View e,f) represent the histological image of the transverse section to show; the flattened conical (CFp) and pointed conical filiform papillae (PFp) and keratin layer (red arrowheads) on the dorsal surface, and ventral surface (VE), and taste buds (TB), transverse muscles bundles (TMB), longitudinal muscles bundles (LMB), connective tissue core (CTC). View (e) by ×40 with H&E stain, while View (f) by ×400, with Masson's Trichrome [Color figure can be viewed at wileyonlinelibrary.com]

fore-tongue occupied the most part of the tongue which is characterized by presence of tapering and biforked lingual tip. The mid-tongue is the second lingual part that attached ventrally with the floor of the mouth through the lingual frenulum. The hind-tongue is the third lingual part that ends with a two long lingual wings. These lingual wings extended posteriorly to form the lateral boundaries of the laryngeal mound (Figure 1a).

3.1.2 | Scanning electron microscopic investigation

SEM investigation of the dorsal surface of the fore tongue terminated by long bifurcated apex. Every bifurcated part exhibited smooth with few detached cells without any papillae (Figure 2a,b). Moreover, the intercellular borders and numerous scattered taste buds are observed on the cell surface by using high magnification (Figure 2c,d). While the SEM investigation of the ventral surface of the fore-tongue revealed the presence of smooth surface with detached keratin layer, as well as the appearance of transverse ridges and few openings of mucous cells (Figure 3a,b).

SEM investigation of the dorsal surface of the midtongue revealed the appearance of the flattened filiform papillae with deciduous superficial cells. These papillae had a serrated border with numerous tiny microridges and few taste buds on their surface (Figure 4a-c). The orifices of the lingual glands are observed by using high magnification (Figure 4d).

SEM investigation of the hind-tongue showing that their dorsal surface had smooth surface with numerous taste buds and openings of the posterior lingual glands without any papillae (Figure 5a,b). Moreover, the SEM investigation of the lateral margins of the hind-tongue carried numerous large and elongated lingual papillae which are arranged in transverse pattern. These papillae have highly serrated ends and imbricate toward the posterior direction (Figure 5c). By high SEM magnification, the intercellular borders and numerous openings of the posterior lingual glands were observed on the cell surface (Figure 5d).

SEM investigation of the dorsal surface of the laryngeal mound showed the presence of numerous cilia with many orifices of the laryngeal glands. In addition, the borders of cells and microridges and few taste buds are well observed (Figure 6a,b). SEM investigation of

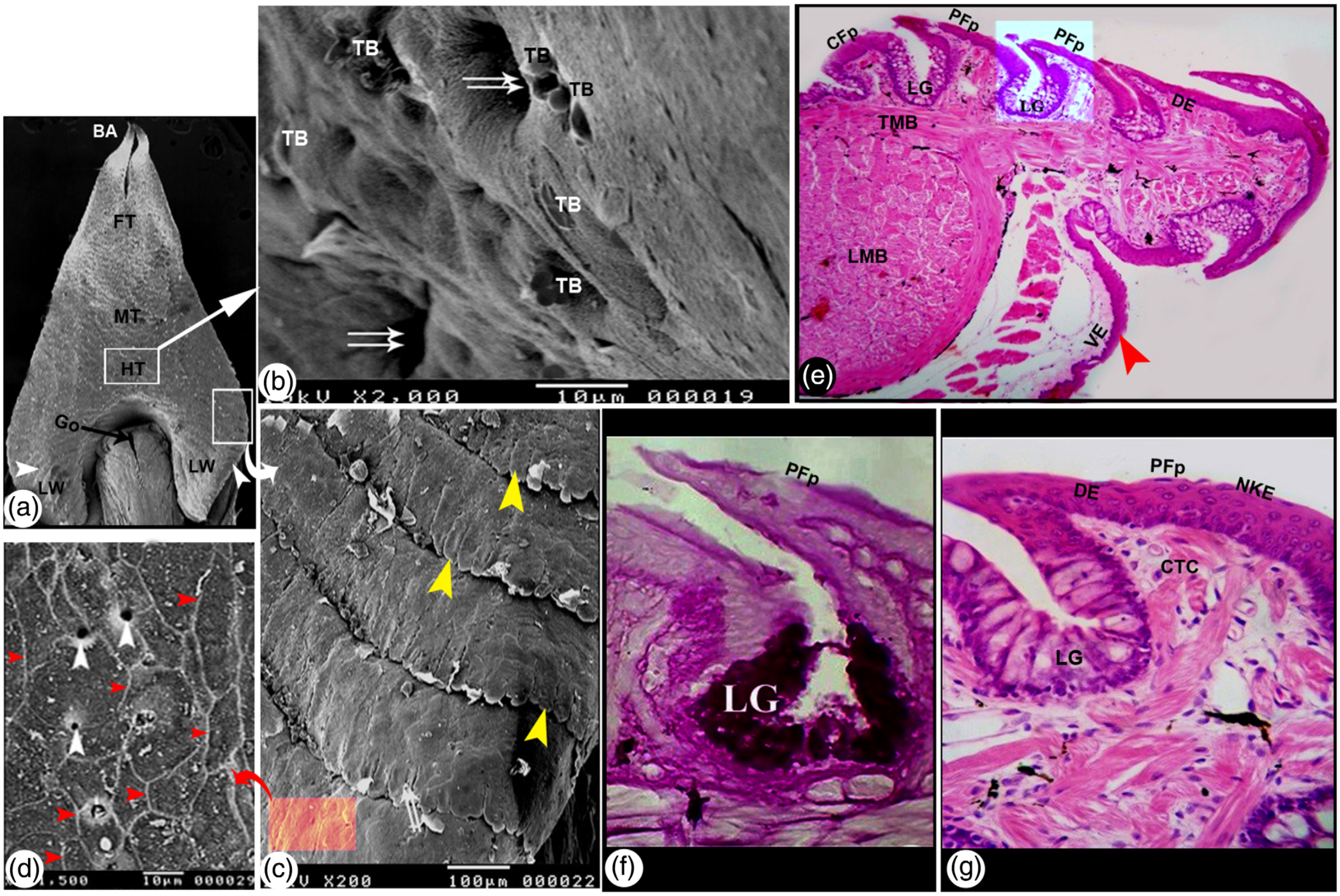

FIGURE 5 Morphological images of the hind-tongue in the Bosc's fringe-toed lizard *Acanthodactylus boskianus*. (View a,b) represent the SEM images of the dorsal surface of the hind-tongue to show; Fore-tongue (FT) with bifurcated apex (BA), Mid-tongue (MT), Hind-tongue (HT) with lingual wing (LW) and laryngeal mound (LM) with median glottis (Go) and lateral margin of the lingual wing (white arrowheads) that had wide orifices of the lingual glands (double white arrows) and taste bud (TB) on the papillary surface. (View c,d) represent the SEM images of the lateral margin of the lingual wing showing rows of the transverse arranged lingual papillae with serrated ends (yellow arrowheads) with numerous orifices of the lingual glands (white arrowheads) and intercellular borders (red arrowhead) on the papillary surface. (View e–g) represent the histological images of the transverse section through the hind-tongue to show the long pointed conical papillae (PFp) and the conical papillae (CFp), and lingual gland (LG), and non-keratinized stratified squamous epithelium (NKE), connective tissue core (CTC), transverse muscles bundles (TMB), longitudinal muscles bundles (LMB). View F represent the secretion of lingual gland contains neutral mucopolysaccharide (LG). View (e) (×100 with H&E), View (f) (×400 with H&E), while View (g) (×400 with PAS-reaction) [Color figure can be viewed at wileyonlinelibrary.com]

the lingual frenulum exhibited many mucosal folds on the smooth surface (Figure 6d).

3.1.3 | Histological investigation

The histological investigation of the fore-tongue of *A. boskianus* revealed that the dorsal and ventral surfaces are covered by stratified squamous epithelium (Figure 2e–g). The mucosa of the dorsal surface of the tongue grown out to form the flattened and conical filiform lingual papillae and their core is filled with loose collagenous connective tissue of submucosal layer (Figure 2f). The mucosa of the ventral surface of the fore-tongue is covered by thin keratinized layer which disappears on the ventral fissure (medial sulcus) (Figure 2g). In addition, the mucosa of the ventral surface interdigitated with the submucosal layer through short projections of loose collagenous connective tissue. Moreover, the submucosal layer is characterized by the presence of bundles of longitudinal and transverse muscle fibers (Figure 2e,f).

The mid-tongue revealed that the dorsal surface is covered by keratinized stratified squamous epithelium, while the ventral surface is covered by non-keratinized stratified squamous epithelium (Figure 4e,f). The dorsal epithelium of the mid-tongue is thicker than that of the ventral one. Moreover, the mucosa of the dorsal surface projected outside to form the flattened and conical filiform papillae which are characterized by a moderate size and inlaid with the taste buds (Figure 4f). The submucosal layer is invaded with muscle bundles of longitudinal and transverse muscle fibers (Figure 4e,f).

The hind-tongue showed that the dorsal surface is covered by non-keratinized stratified squamous epithelium which projected outside to form long pointed conical and conical filiform papillae (Figure 5e). Alveoli of the lingual gland are located between the filiform papilla (inter-papillary pit) (Figure 5g). The secretions of these

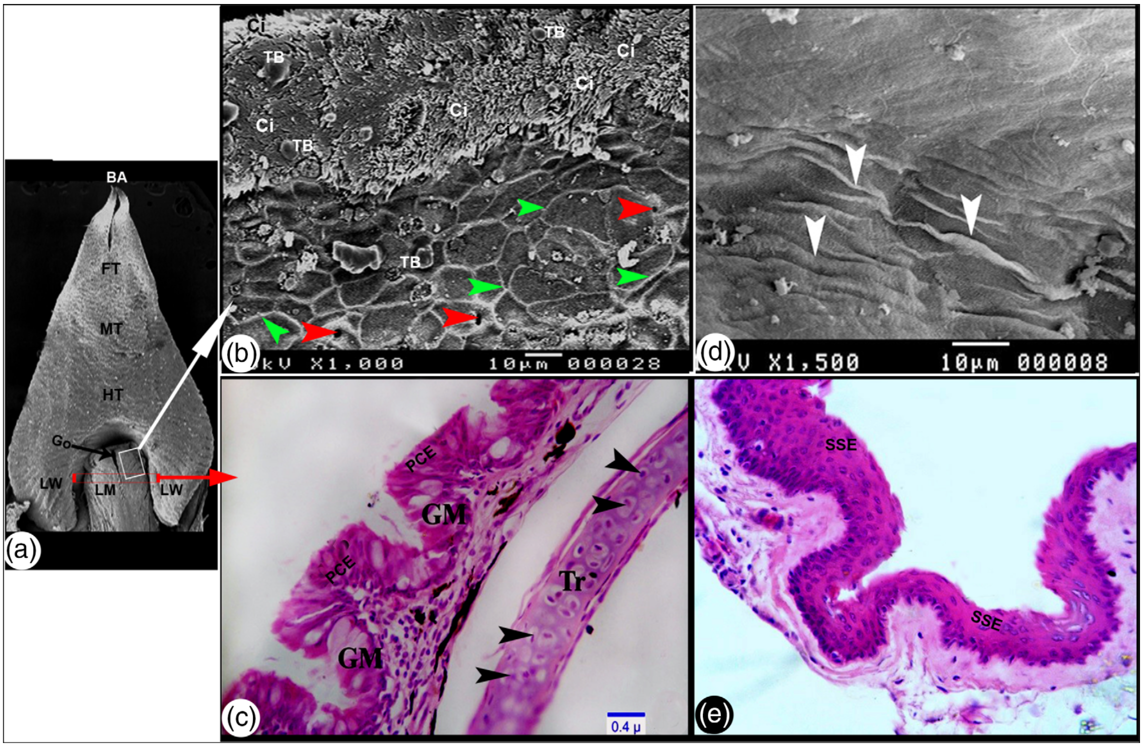

FIGURE 6 Morphological images of the lingual wings and laryngeal mound in the Bosc's fringe-toed lizard *Acanthodactylus boskianus*. (View a,b) represent the SEM images to show; Fore-tongue (FT) with bifurcated apex (BA), Mid-tongue (MT), Hind-tongue (HT) with lingual wing (LW) and laryngeal mound (LM) with median glottis (Go), numerous of cilia (Ci), orifices of the laryngeal glands (red arrowheads), and appearance of intercellular borders (green arrowheads), and taste bud (TB). (View c) represent the transverse histological sectional image through the mucosa of laryngeal mound that composed of pseudostratified ciliated columnar epithelium (PCE), numerous tubular laryngeal glands (GM), the chondrocytes lodged within the lacunae (black arrowheads) and trachea (Tr). (View d) represent the SEM images of the lingual frenulum showing; the smooth surface with many folds (white arrowhead). (View e) represents the transversal histological sectional images through the lingual frenulum showing the transitional-like epithelium with rounded nuclei and stratified squamous epithelium with flattened nuclei (SSE). View c and e by ×400 with H&E [Color figure can be viewed at wileyonlinelibrary.com]

glands contain a large amount of mucous which gives positive with PAS reaction (Figure 5f).

The laryngeal mound revealed that this part is covered by pseudostratified ciliated columnar epithelium incubate numerous tubular laryngeal glands open directly on the surface of the laryngeal mound and tongue (Figure 6c). The lingual frenulum is covered by transitional-like epithelium which similar to the squamous epithelium when it is stretched with flattened nuclei, but when it is collapsed the nuclei of the more superficial cells becomes rounded (Figure 6e).

3.2 | Sinai fan-fingered gecko *Ptyodactylus guttatus*

3.2.1 | Morphological investigation

The tongue of *Ptyodactylus guttatus* is voluminous, fills the most buccal cavity with length (~0.8 mm) and occupies the half length of the mandible (~1.6 mm). *P. guttatus* had a slightly triangular shaped tongue with round apex and broad base. The mid-tongue attached ventrally with the floor of the mouth through the lingual frenulum. Grossly, the lingual apex was slightly bifurcated. The hind-tongue terminated with two short lingual wings (Figure 1b).

3.2.2 | Scanning electron microscopic investigation

SEM investigation along the dorsal surface of the fore-tongue showed that it had a slightly bifurcated apex that bordered rostrally by the anterior border of the oval bodies that appear clearly on the ventral surface. The ventral lingual surface had a median sulcus that represents the posterior extension of the slightly lingual apex bifurcation. The SEM magnification exhibited the presence of numerous pentagonal and hexagonal shaped cylindrical and conical papillae (Figure 7a–c). The high SEM magnification exhibited the borders of cells, tiny microridges and few apertures of taste buds (Figure 7c).

Moreover, the ventral surface of the mid-tongue clarify that the slightly bifurcated apex bordered laterally by oval slightly projected smooth body that surrounded posteriorly and medially by a shallow semicircular groove (Figure 3c). By high magnification, every oval body carried numerous semi-like scutate papillae. These papillae are

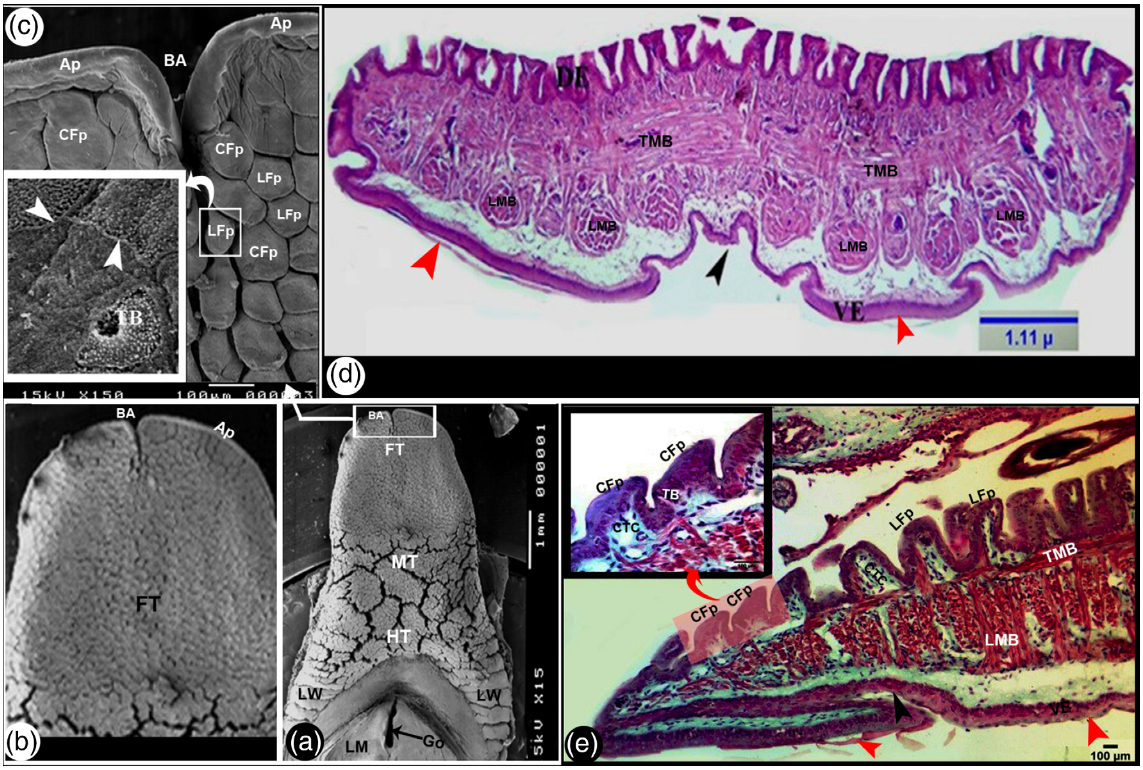

FIGURE 7 Morphological images of the fore-tongue in the Sinai fan-fingered gecko *Ptyodactylus guttatus*. (View a–c) represent the SEM images to show; Fore-tongue (FT) with shallow bifurcated apex (BA) and the conical (CFp) and cylindrical filiform papillae (LFp), anterior pad (AP) and the intercellular borders (white arrowhead) and taste buds (TB). Mid-tongue (MT), Hind-tongue (HT) with lingual wing (LW) and laryngeal mound (LM) with median glottis (Go). (View d) represent the histological image of the transverse section through the fore-tongue to show the dorsal epithelium (DE) and ventral epithelium (VE), ventral sulcus (black arrowhead), keratin layer (red arrowheads), transverse muscles bundles (TMB), longitudinal muscles bundles (LMB). By ×40 with H&E. (View e) represent the histological image of the longitudinal section through the fore-tongue to show; conical (CFp) and cylindrical filiform papillae (LFp), connective tissue core (CTC), deciduous keratin (red arrowheads), ventral epithelium (VE), transverse muscles bundles (TMB), longitudinal muscles bundles (LMB). By ×100 with H&E. Upper left show the appearance of taste buds (TB). By ×400 with Masson's Trichrome [Color figure can be viewed at wileyonlinelibrary.com]

arranged in overlapping rows and directed backwardly, in addition, carry many knobs on its surface (Figure 3d).

SEM investigation along the dorsal surface of the mid-tongue exhibited wide distribution of the conical and cylindrical filiform papillae (Figure 8a,b). The cylindrical filiform papillae are exhibited the smooth surface with a border of cells and perforated by numerous taste buds (Figure 8c), while the conical filiform papillae are exhibited the rough surface with a border of cells, and numerous micro-spines and perforated by few taste buds (Figure 8d).

SEM investigation of the ventral surface of the mid-tongue exhibited numerous transverse ridged mucosal folds. In addition, with magnification, the transverse ridged mucosal folds had a less clear cellular borders and numerous knobs. Moreover, with high magnification the mucosal folds had numerous microridges and numerous knobs (Figure 9a). SEM investigation of the lingual frenulum exhibited the presence of the smooth surface with prominent mucosal folds with less deciduous cells and a very shallow cellular borders (Figure 9c).

SEM investigation of the dorsal surface of the hind-tongue exhibited many wide orifices of the posterior lingual gland and clearly observed numerous clear intercellular borders (Figure 10a,b). SEM investigation of the laryngeal mound exhibited the dense appearance of the posterior directed cilia and orifices of the laryngeal glands (Figure 11a,b).

3.2.3 | Histological investigation

Histological investigation of the tongue revealed that the dorsal and ventral surfaces of the fore-tongue are covered by stratified squamous epithelium (Figure 7d,e). The dorsal surface of the most fore-tongue is covered by non-keratinized stratified squamous epithelium. That epithelium forms downward folds (retridges) which interdigitate with the upper projections of the submucosal constructing the conical filiform and cylindrical papillae. These papillae are perforated by few taste buds. The submucosal is formed from dense collagenous connective tissue with the distribution of the longitudinal and transverse muscular bundles (Figure 7e). On the other hand, the ventral surface is covered by thick keratinized layer (Figure 7d,e/red arrowheads), while the ventral fissure is covered by non-keratinized squamous epithelium (Figure 7d,e), black arrowheads).

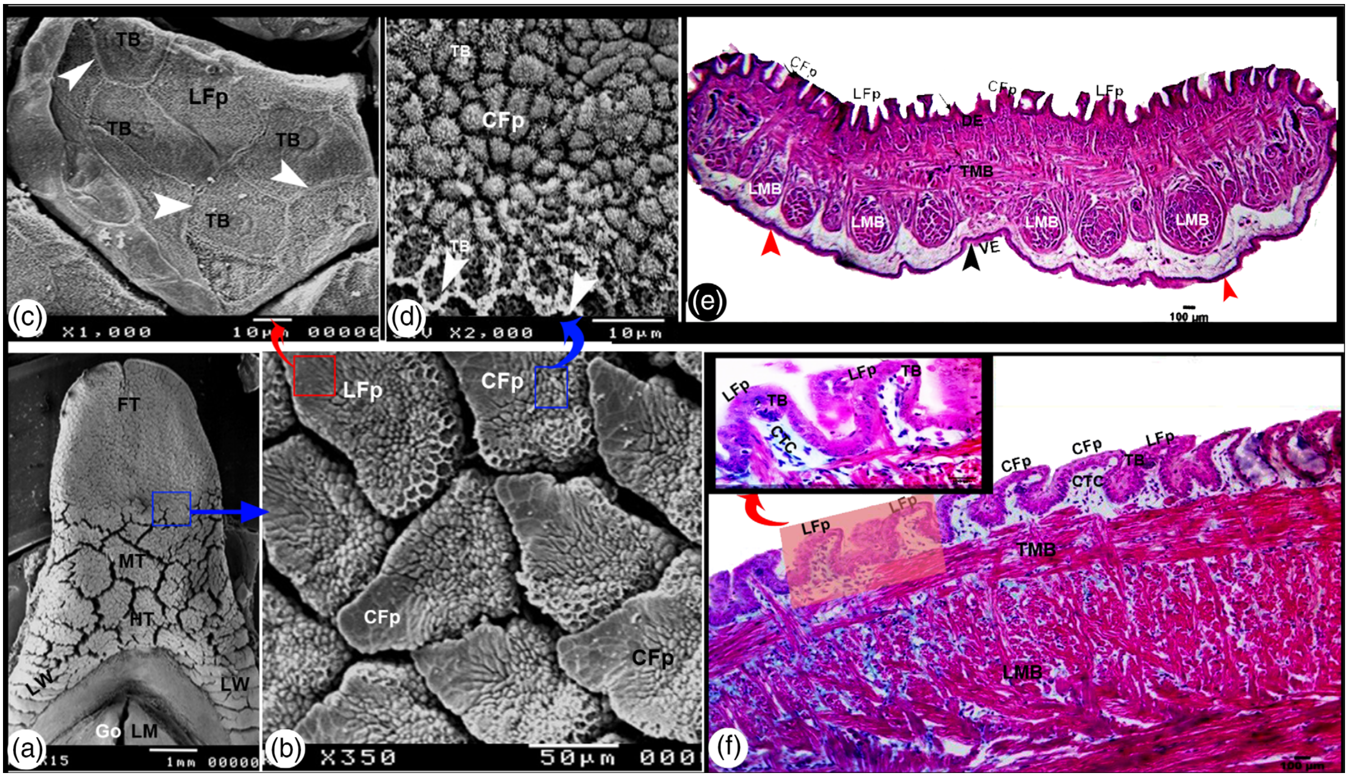

FIGURE 8 Morphological images of the mid-tongue in the Sinai fan-fingered gecko *Ptyodactylus guttatus*. (View a–d) represent the SEM images to show; Fore-tongue (FT), Mid-tongue (MT) with the conical (CFp) of numerous spinated tubercles and cylindrical filiform papillae (LFp) of smooth surface, the intercellular borders (white arrowhead) and taste buds (TB). Hind-tongue (HT) with lingual wing (LW) and laryngeal mound (LM) with median glottis (Go). (View e) represent the transversal histological sectional image through the mid-tongue to show; dorsal epithelium (DE) had the conical (CFp) and cylindrical filiform papillae (LFp) and ventral epithelium (VE), transverse muscles bundles (TMB), longitudinal muscles bundles (LMB), ventral sulcus (black arrowhead), and keratin layer (red arrowheads). By ×40 with H&E. (View f) represent the longitudinal histological sectional image through the hind-tongue showing the conical (CFp) and cylindrical filiform papillae (LFp) with taste buds (TB), connective tissue core (CTC), transverse muscles bundles (TMB), longitudinal muscles bundles (LMB). By ×100 with Masson's Trichrome and the upper View by ×400 with Masson's Trichrome [Color figure can be viewed at wileyonlinelibrary.com]

The dorsal surface of the mid-tongue is covered by stratified squamous epithelium which formed the conical filiform and cylindrical papilla (Figure 8e,f). Moreover, the mucosa of the dorsal surface interdigitated with the underlying submucosal layer, which is formed of the dense collagenous fibers, to form the core of papillae. The muscle bundles invade the core of these papillae (Figure 8e,f). The ventral surface of the mid-tongue is covered by thin layer of non-keratinized stratified squamous epithelium which converted into a transitional epithelium in some region and folds (Figure 9b). The lingual frenulum is covered by a transitional-like epithelium resembles that of the *A. boskianus* (Figure 9d).

The dorsal surface of the hind-tongue is covered by non-keratinized stratified squamous epithelium with tall filiform papillae and many tubular lingual glands (Figure 10c,d). The lingual glands gave a positive reaction with PAS stain which means that their secretion contains large amounts of mucous substances (Figure 10c).

Histological investigation of the laryngeal mound revealed that its dorsal surface is covered by pseudostratified ciliated columnar epithelium incubi simple tubular glands (Figure 11c,d). These simple tubular glands react positively with PAS stain which indicated that their secretion contains a large amount of mucous substances (Figure 11d).

The main variations between the lingual papillae and their keratinization at the different lingual parts between the two reptilian species in the current study were summarized in Table 1.

4 | DISCUSSION

The morphological properties of the tongues of two studied species; *A. boskianus* and *P. guttatus* exhibited the presence of great variations related to their function during prey capture. Both studied species depend on a fast bite to seize their prey by using their jaws. Although their jaws provided with small teeth but it cannot penetrate the skin of the prey, thus these reptile species are using different strategies during catching their prey and continue by several protraction–retraction motions of the tongue (Jamniczky, Russell, Johnson, Montuelle, & Bels, 2009). *A. boskianus* (Lizard) uses active search strategy, while *P. guttatus* (Gecko) uses sit-and-wait strategy to find their food (Saber et al., 1994). These different feeding strategies affected in the

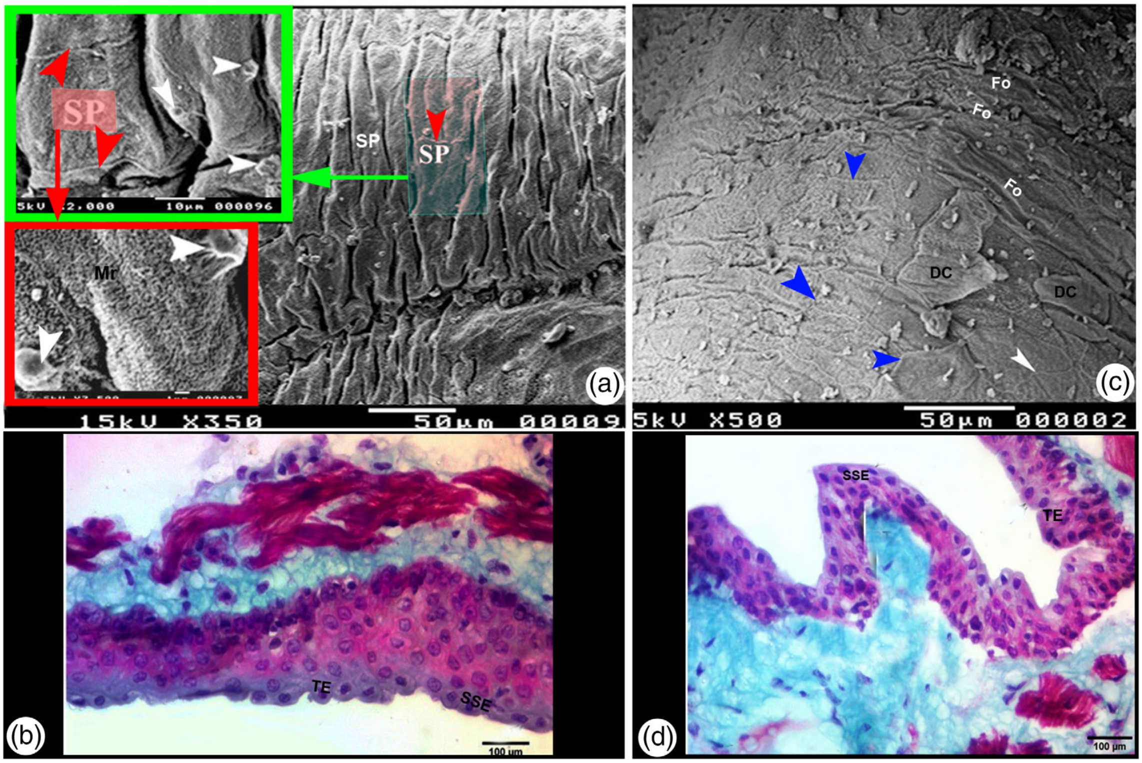

FIGURE 9 Morphological images of the mid-tongue in the Sinai fan-fingered gecko *Ptyodactylus guttatus*. (View a) represent the SEM image of the ventral surface of the mid-tongue to show; the thick mucosal folds (SP), the borders of cells (red arrowheads) and appearance of numerous knobs (white arrowheads). (View c) represent the SEM image of the lingual frenulum to show; the surface is smooth with shallow prominent folds (Fo), less deciduous cells (DC) and observed borders of cells (blue arrowhead). (View b) represent the longitudinal histological sectional histological image to show the ventral epithelium just behind the lingual nail is composed of stratified squamous epithelium (SSE), microridges (Mr), and Transitional one (TE). (View d) represents the longitudinal histological sectional image through the lingual frenulum to show it is composed of Transitional epithelium (TE), and squamous stratified epithelium (SSE) (×400, Masson's Trichrome) [Color figure can be viewed at wileyonlinelibrary.com]

structure and appearance of the tongues of these examined species. The main variations between the lingual papillae and their keratinization at the different lingual parts between the two reptilian species in the current study were summarized in Table 1.

The tongue of *A. boskianus* had narrow-tapering apex with slightly broad base as well as its lingual apex possess a deep bifurcation while, the tongue of *P. guttatus* with broad rounded tip with slightly small not clear bifurcation that may described as just a narrow small fissure appears clearly on the ventral surface of its apex. The narrow tapering shape of the tongue in *A. boskianus* may help to increase speed of protraction movement. Moreover, the forked tongue in *A. boskianus* may provide more surfaces available for sensory function during active searching as reported in *Stenodactylus petrii* (Darwish, 2012; El-Mansi et al., 2020).

According to the previously published data, the structural pattern of the bifurcation tongue is represented in different reptiles species, such as *Gecko japonicas* (Iwasaki, 1990), *Anoliscaro linensis* (Rabinowitz & Tandler, 1991), *Oplurus cuvieri* (Delheusy, Toubeau, & Bels, 1994), and *Pogona vitticeps* (Schaerlaeken, Meyers, & Herrel, 2007). However, this phenomenon of bifurcation of the tongue is not restricted on the reptile species but also found in some bird like prey–predator bird, for example, Hume's tawny owl *Strix butleri* (Abumandour & El-Bakary, 2017b), house sparrow *Passer domesticus* (Abumandour, 2018) and white tailed eagle (Jackowiak & Godynicki, 2005), and in mammals (Kienle, Ekdale, Reidenberg, & Demere, 2015; Massoud & Abumandour, 2019). These previous observations are confirmed that the bifurcation in tongues of lizards may facilitate the swallowing process of prey in agreement with conclusion of (Schwenk, 2000). As well as the tongue is a highly versatile system performing a wide diversity of functions such as prey capture or manipulate chemoreception and drinking.

Meanwhile, the broad tongue in *P. guttatus* may provide wide surface available to loading the water during drinking process or carry its prey backward to buccal cavity. However, in *A. boskianus* and *P. guttatus*, the fore-tongue is protruded frequently outside the jaw to touch and tasting the substrate (food or water), the frequency of this motion in *A. boskianus* is more than that in *P. guttatus*. Tongue length of *A. boskianus* relative to the length of the mandible was more than that in *P. guttatus* which help to more flicking. (Cooper, 1994) stated tongue-flicking is the essential squamate behavior. The reptiles tongue serves as an environmental sampler and delivery device to the vomeronasal (Jacobson's) organ

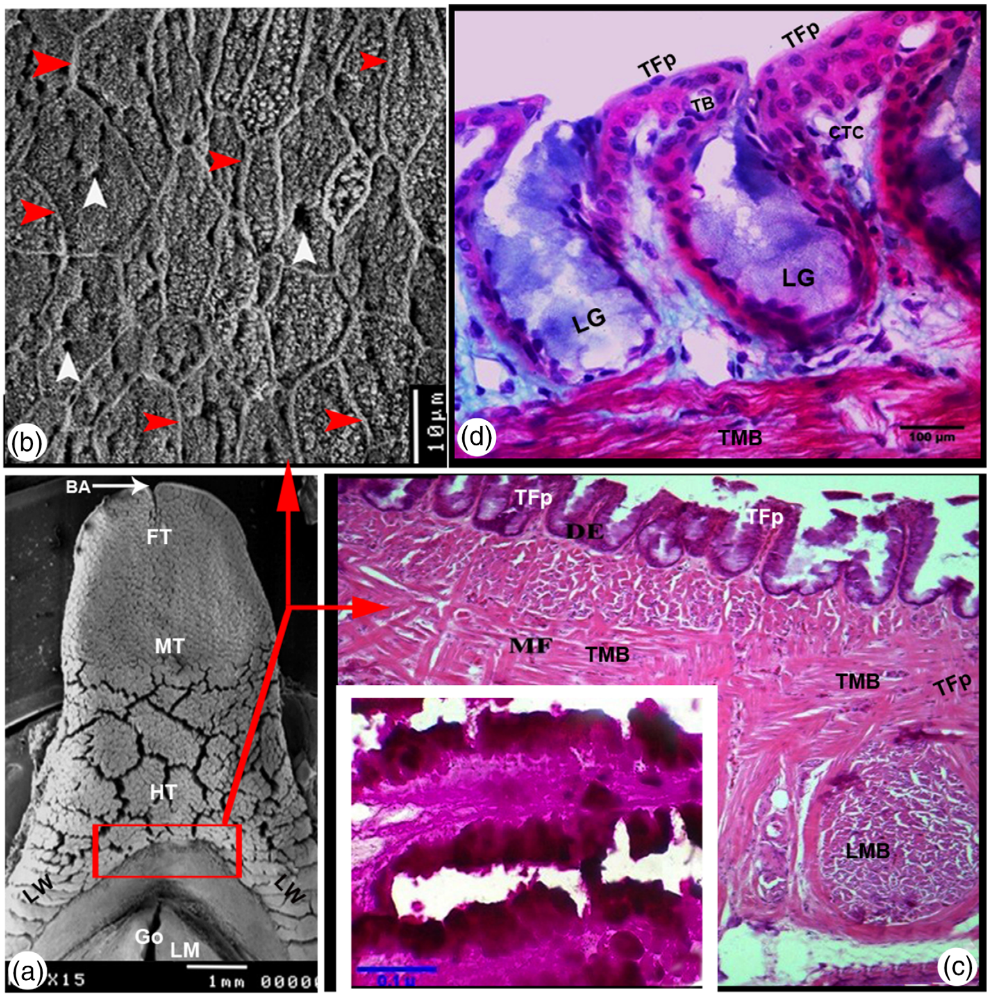

FIGURE 10 Morphological images of the hind-tongue in the Sinai fan-fingered gecko *Ptyodactylus guttatus*. (View a,b) represent the SEM images to show; Fore-tongue (FT), Mid-tongue (MT), Hind-tongue (HT) numerous orifices of lingual gland (white arrowheads) and clearly observed borders of cells (red arrowheads). Lingual wing (LW) and laryngeal mound (LM) with median glottis (Go). (View c,d) represent the histological image of the hind-tongue showing the dorsal epithelium (DE) with tall filiform papillae (TFP) and muscle fibers (MF) with transverse muscles bundles (TMB), longitudinal muscles bundles (LMB), connective tissue core (CTC), taste buds (TB), and lingual glands (LG). The lower left magnified image in the (View c) shows the natural of secretion of lingual gland (×400, PAS-reaction). View c by ×40 with H&E stain, while View d by ×400 with Masson's trichrome stain [Color figure can be viewed at wileyonlinelibrary.com]

found in the roof of the mouth or in the nasal passages (El-Mansi et al., 2020; Filoramo & Schwenk, 2009).

The present study assumed that the reptiles' species that use a sit-and-wait strategy like *P. guttatus* are thought to rely on visual cues primarily, while actively hunting species like *A. boskianus* would predominantly use the chemical information, in agreement with view of Cooper (1998) and confirmed by Baeckens et al. (2017) who concluded that the morphology of the tongue and the vomeronasal-organs is believed to mirror this dichotomy as well as the reptilian species using chemical signals to communicate with heterospecifics or conspecifics.

The present study indicated variability of keratinization along the dorsal surface of the mid-tongue of *A. boskianus* and ventral surface of the fore-tongue of both *A. boskianus* and *P. guttatus*. That keratin layer which covers the ventral surface of the fore-tongue is thick and well-developed in *P. guttatus* in comparison with that of *A. boskianus*. The thick keratin layer in *P. guttatus* support the mechanical bending of the free portion of the tongue, while the keratin layer in *A. boskianus* resisting abrasive force to protect the soft epithelium during mechanical perform of the tongue.

Disappearance of keratinization on the dorsal lingual epithelium is common feature in some reptiles (Abbate et al., 2010; Al-Zahaby, Nasr, & Hassan, 2018; Herrel et al., 2005), while Wassif (2001) recognized that keratinization is represented at the tip of the tongue of Scincine Lizard, while El-Mansi et al. (2020) in *Psammophis sibilans* observed the dorsal and ventral lingual surface were covered by the kertainized material but the degree of keratinization reached to three times on the dorsal than the ventral lingual surface. Similar results were described by Iwasaki and Miyata (1985) and Iwasaki (1990) in Japanese lizard *Gecko japonicas*.

Moreover, the surface of non-keratinized cells provides with tiny microridges on the mid and hind-tongue of *A. boskianus* while these microridges appear only on the hind-tongue of *P. guttatus*. The smooth surface of the fore-tongue in both studied reptile-species may facilitate the slipping process of prey into esophagus. Beisser, Lemell, and Weisgam (2004), Iwasaki and Kobayashi (1992), and Wassif (2002) reported the appearance of microridges on the buccal epithelia aid in spreading the mucosal-film that produced by the lingual gland over the cellular surface to decrease friction forces occur on this region.

The present study recognized different types of papillae along the dorsal surface of the tongue of both studied species; in *A. boskianus* the flattened and conical papillae appear along the whole length of the free portion of tongue, whereas the long conical filiform papillae are formed by the epithelium of hind-tongue. While in *P. guttatus*, the

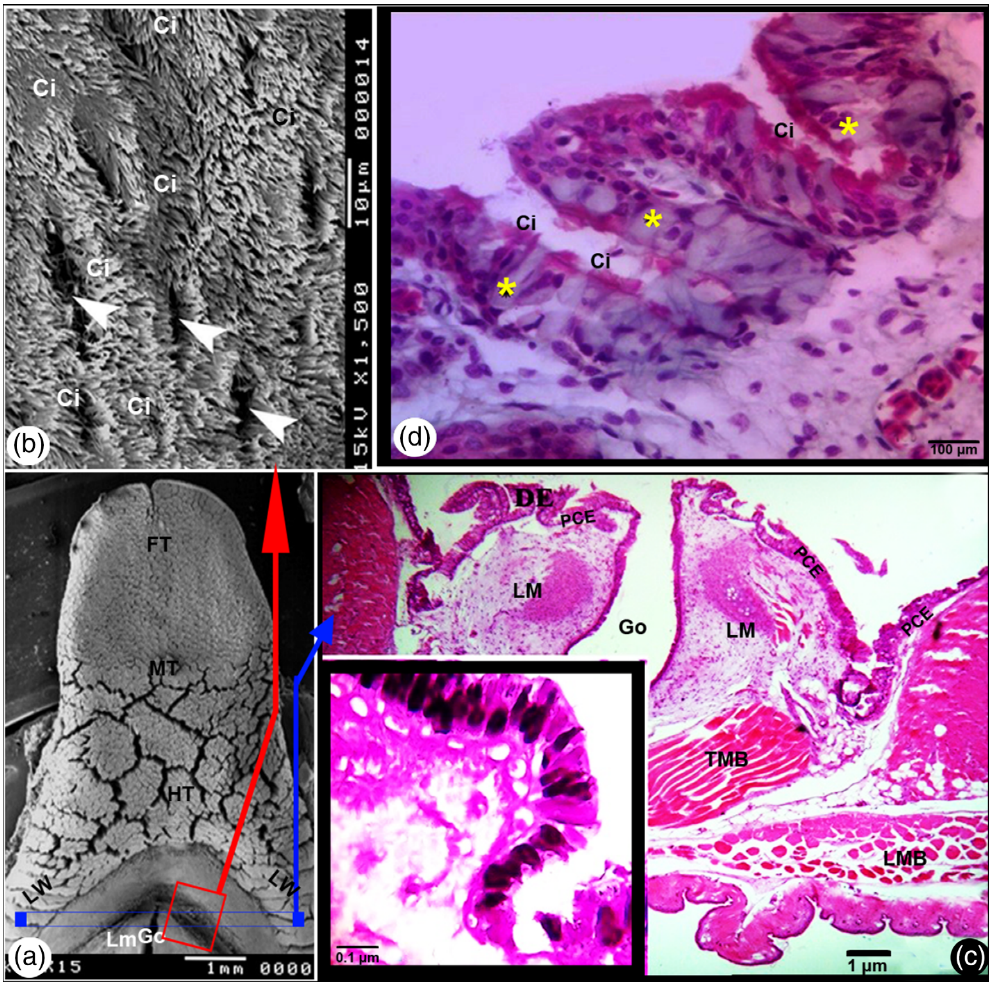

FIGURE 11 Morphological images of the laryngeal mound in the Sinai fan-fingered gecko *Ptyodactylus guttatus*. (View a,b) represent the SEM images to show; Fore-tongue (FT), Mid-tongue (MT), Hind-tongue (HT) with lingual wing (LW) and laryngeal mound (LM) with median glottis (Go), the surface provides with numerous of cilia (Ci) and appearance orifices of laryngeal gland (white arrowhead). (View c) represent the histological image of the transverse section of the hind-tongue to show the pseudostratified ciliated columnar epithelium (PCE) covers the dorsal surface (DE) of laryngeal mound (LM) with median glottis (Go), transverse muscles bundles (TMB), longitudinal muscles bundles (LMB). The lower left magnified image shows the secretion of laryngeal gland contains neutral mucopolysaccharides (purple color) by ×100 with PAS-reaction. View c by ×40 with H&E stain. (View d) represent the histological image of the longitudinal section of the hind-tongue to show the cilia (Ci) and laryngeal gland (yellow star). By ×400 with Masson's trichrome stain [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 The main variations between the lingual papillae and their keratinization at the different lingual parts between the two reptilian species in the current study

	Acanthodactylus boskianus			*Ptyodactylus guttatus*		
	Fore-tongue	**Mid-tongue**	**Hind-tongue**	**Fore-tongue**	**Mid-tongue**	**Hind-tongue**
Type of lining epithelium (dorsal surface)	Non-keratinized stratified squamous epithelium	Keratinized stratified squamous epithelium	Non-keratinized stratified squamous epithelium	Non-keratinized stratified squamous epithelium		
Lingual papillae and glands	Flattened conical filiform papillae		• Long pointed conical • Conical filiform papillae • Many tubular lingual glands	• Conical filiform papillae Cylindrical papillae		• Tall filiform papillae • Many tubular lingual glands
Taste buds	Numerous scattered taste buds	Few taste buds	Numerous taste buds	Few taste buds	Numerous taste buds	Absent

conical filiform and cylindrical papillae exhibited on the fore-tongue and mid-tongue, whereas tall filiform papillae are formed on the hind-tongue. The different types of lingual papillae have been recognized in different reptile-species such as; in Chameleon (Fouda, Sabry, & Abou-Zaid, 2015), Varanous niloticus niloticus (Al-Zahaby et al., 2018), gecko Oplurus cuvieri (Delheusy et al., 1994), and lizard (Taha, 2013). However, the absence of the lingual papillae from the dorsal lingual surface were reported in some turtle and snake (El-Mansi et al., 2020; Iwasaki & Kumakura, 1994; Moa, Wang, Huang, Chao, & Chen, 1991) and suggested that snake tongue does not appear to be important for the direct intake of food.

According to the present study, the variations of the papillae among their size, shape and distribution along the free portion may function as mechanical support for the food manipulation. In addition, the interpapillary space that located between these papillae act as a pouch to pick up a large amount of any materials contacting with the surface of tongue during its protrude to collecting airborne and substrate-fixed chemical particles then retracts and transported to vomeronasal organ. This may explain why the numerous of taste buds are observed along the two-third of the tongue of *P. guttatus*, while few restricted along the fore-tongue of *A. boskianus* (lizard) but increase on its mid-tongue. Jamniczky et al. (2009) noted that gekkota largely lack taste buds and concluded that this phenomenon may be phylogenetic and also may be a functional or adaptive significance to it. This view disagree with that mentioned by Bayoumi et al. (2011), who observed taste buds on the apical and lateral surfaces of the dome-shaped lingual papillae on the tip of *P. guttatus*, while they are completely absent on the papillae of *A. boskianus* tongue. Taha (2013) observed the presence few taste buds in the epithelium of the three parts of the tongue in *Trachylepis vittata*.

The presence of taste buds on the tongue tip may play an important role in receiving chemical information of food (Nasr, Gamal, & Elsheikh, 2012). Our results confirm that the tongue of *A. boskianus* is used as a chemoreceptor organ to follow the pheromone trails of prey and mates, while in *P. guttatus*, its tongue is directly connecting with the prey for long time more than in *A. boskianus*, therefore, that allows to receive more chemical and mechanical information about this prey and this occur through the taste buds that scattered on their parts of tongue.

5 | CONCLUSION

Obviously, the tongue *A. boskianus* possess many characteristics, for example, bifurcation the anterior tip, few appearance of taste buds on fore-tongue but increase on mid-tongue, that confirm this lizard is using its tongue to collect more information about their prey in air before touch it, so this tongue of lizard acts as a chemoreceptor organ. In contrast, in *P. guttatus*, its tongue is directly connecting with the prey for long time, consequently, that allows receiving more chemical and mechanical information about this prey and this occur through the taste buds that scattered on their parts of tongue.

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CONFLICTS OF INTEREST

All authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data Availability Statement: Author elects to not share data

ORCID

Fatma A. Mahmoud https://orcid.org/0000-0002-1608-3539
Mohamed M. A. Abumandour https://orcid.org/0000-0002-3289-1982
Ramadan M. Kandyel https://orcid.org/0000-0001-6516-2148

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